COMPARISON BETWEEN REVERSE AND TRADITIONAL SCREENING ALGORITHMS FOR SYPHILIS DIAGNOSIS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

DR NURUL AZIRA BINTI SIDEK

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PATHOLOGY (MEDICAL MICROBIOLOGY)

SCHOOL OF MEDICAL SCIENCES

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SUPERVISOR: DR NABILAH ISMAIL

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Symbols/ abbreviations	Meaning
%	Percentage
≥	Greater than or equal to
-	To or minus
/	Division
<	Less than
μL	Microliter
℃	Degree Celcius
+	Addition
x	Times or multiplication
AIDS	Acquired immunodeficiency syndrome
COI	Cut-off index
CSF	Cerebrospinal fluid
DFA	Direct fluorescent antibody
DNA	Deoxyribonucleic acid
ECLIA	Electrochemiluminescence immunoassay
HIV	Human immnudeficiency virus
HUSM	Hospital Universiti Sains Malaysia
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MSM	Men who have sex with men
NAAT	Nucleic acid amplification test

Symbols/ abbreviations	Meaning
NPV	Negative predictive value
PCR	Polymerase chain reaction
PPV	Positive predictive value
ROC	Receiver operating characteristics
RPR	Rapid plasma reagin
STI	Sexually transmitted infection
subsp.	Subspecies
T. pallidum	*Treponema pallidum*
TpN	*Treponema pallidum* antigen
TPPA, TP·PA	*Treponema pallidum* particle agglutination
VDRL	Venereal disease research laboratory

ABSTRAK

Perbandingan antara algoritma saringan terbalik dan tradisional untuk diagnosis sifilis di Hospital Universiti Sains Malaysia

Pengenalan: Kejadian sifilis global meningkat secara drastik di seluruh dunia, termasuk Malaysia. Algoritma saringan dengan kepekaan dan pengkhususan tinggi harus ada untuk mengesan sifilis dengan tepat. Kajian ini bertujuan untuk menilai prestasi algoritma saringan terbalik untuk diagnosis sifilis pada populasi kita. Kami membandingkan ketepatan diagnostik antara dua kaedah pemeriksaan yang tersedia: pemerikasaan imun elektrokimia (ECLIA) dan reagin plasma cepat (RPR), masing-masing mewakili algoritma penyaringan terbalik dan tradisional.

Tatacara: Sebanyak 206 sampel serum dimasukkan dalam kajian ini. Sampel ini diuji dengan ujian ECLIA, RPR, dan penyatuan zarah *Treponema pallidum* (TPPA). TPPA dianggap sebagai ujian rujukan standard. Algoritma saringan terbalik dan tradisional diterapkan pada semua spesimen. Sensitiviti, kekhususan dan nilai ramalan ujian saringan dikira. Analisis keluk ROC digunakan untuk menentukan bacaan RPR optimum yang berkaitan dengan kereaktifan TPPA.

Keputusan: Dari 206 sampel serum, 32 (15.53%) didiagnosis menghidap sifilis menggunakan algoritma terbalik, tetapi hanya 23 (11.17%) yang didiagnosis menghidap sifilis menggunakan algoritma tradisional. Sebilangan besar kes sifilis adalah lelaki dan berumur 50 tahun keatas. Mengikuti algoritma terbalik, 27 (13.11%)

kes menunjukkan keputusan ECLIA dan RPR yang tidak selari. Ujian lebih lanjut dengan TPPA mendedahkan bahawa 5 (2.42%) kes adalah ECLIA positif palsu. Sensitiviti, pengkhususan, PPV dan NPV ECLIA dan RPR untuk pengesanan sifilis masing-masing adalah 100%, 97.13%, 86.49%, 100%, dan 71.88%, 92.53%, 63.89%, 94.71%.

Kesimpulan: Algoritma terbalik menunjukkan prestasi yang lebih baik dengan pengesanan sifilis yang lebih tinggi. ECLIA menunjukkan ketepatan diagnostik yang sangat baik sebagai ujian saringan untuk sifilis dengan kadar positif palsu yang rendah menyokong penggunaannya di kalangan penduduk. Kereaktifan RPR (titer ≥1: 1) harus dapat meramalkan kereaktifan ujian pengesahan treponema. Walau bagaimanapun, penilaian lebih lanjut dengan ukuran saiz sampel lebih besar diperlukan.

Kata kunci: Sifilis, algoritma tradisional, algoritma terbalik, ECLIA, TPPA

ABSTRACT

Comparison between reverse and traditional screening algorithms for syphilis diagnosis in Hospital Universiti Sains Malaysia

Introduction: The global incidence of syphilis increased drastically throughout the world, including Malaysia. Screening algorithm with high sensitivity and specificity should be available to detect syphilis accurately. This study aimed to assess the performance of reverse screening algorithm for syphilis diagnosis in our population. We compare the diagnostic accuracy between two available screening methods: electrochemiluminescence immunoassay (ECLIA) and rapid plasma reagin (RPR), each represents reverse and traditional screening algorithms, respectively.

Materials and methods: A total of 206 serum samples were included in this study. These samples were tested with ECLIA, RPR, and *Treponema pallidum* particle agglutination (TPPA) assay. TPPA was considered as the gold standard test. Reverse and traditional screening algorithms were applied to all specimens. Sensitivity, specificity and predictive values of the screening tests were calculated. ROC curve analysis was used to determine optimal cut-off RPR titer related to TPPA reactivity.

Results: Out of 206 serum samples, 32 (15.53%) were diagnosed with syphilis using the reverse algorithm, but only 23 (11.17%) were diagnosed with syphilis using the traditional algorithm. Majority of syphilis cases were male and among ≥ 50 years age group. Following the reverse algorithm, 27 (13.11%) cases showed discordant ECLIA and RPR results. Further testing with TPPA reveals that 5 (2.42%) cases are false-

positive ECLIA. The sensitivity, specificity, PPV and NPV of ECLIA and RPR for syphilis detection were 100%, 97.13%, 86.49%, 100%, and 71.88%, 92.53%, 63.89%, 94.71% respectively.

Conclusion: The reverse algorithm showed better performance with higher syphilis detection. ECLIA revealed excellent diagnostic accuracy as a screening test for syphilis with a low false positivity supporting its use in our population. RPR reactivity (titer ≥1:1) should be able to predict the reactivity of confirmatory treponemal test. However, further evaluation with a larger sample size is required.

Keywords*:* Syphilis, traditional algorithm, reverse algorithms, ECLIA, TPPA

CHAPTER 1: INTRODUCTION

LITERATURE REVIEW

1.1 Background of the study

Syphilis is a curable disease but remains a cause of substantial morbidity and mortality worldwide.[1] Despite the availability of successful antibiotic treatment, syphilis has been a global health issue due to its incidence increased drastically throughout the world over the last decades.[2–4]

The responsible microbial agent of syphilis, *Treponema pallidum* subspecies *pallidum* mainly transmitted through sexual contact, via blood transfusion, or vertical transmission.[1,2,5] The symptoms of early syphilis often go unnoticed.[6] When left undiagnosed and inadequately treated, it can cause a long course of varied clinical manifestations, including neurological, cardiovascular, and other multi-system damage.[7,8] The consequences are serious, even life-threatening.[7–9]

There are various techniques available for syphilis diagnosis; however, serology remains the optimal testing method in many laboratories.[10] Many new serological methods have been developed in recent years, leading to the availability of automation tests for syphilis screening. The use of automation for screening test has changed the traditional screening algorithm for syphilis being reversed.[11,12] In general, the reversed screening algorithm is better in reducing time and cost for syphilis diagnosis and detecting the very early or late syphilis stages.[13,14]

Unfortunately, the use of the reversed screening algorithm has not been widely implemented in Malaysia. Therefore, this study was conducted to assess the

performance of reverse screening algorithm for syphilis diagnosis in our population. We compare the diagnostic accuracy between two available screening methods: electrochemiluminescence immunoassay (ECLIA) and rapid plasma reagin (RPR), each represents reversed and traditional screening algorithms, respectively.

The main issue related to the use of the reverse algorithm is the discrepancy between treponemal screening and nontreponemal confirmatory test results. In order to minimize the need for a second confirmatory treponemal test in the diagnosis of syphilis, studies to evaluate the correlation of the automation treponemal immunoassay signal intensity values with the second confirmatory treponemal tests had been performed.[15] Therefore, in our study, we would like to determine the optimal cut-off for RPR titer, which represents the true-positive second confirmatory treponemal test [*Treponema pallidum* particle agglutination assay (TPPA)]. Feasibly, the findings from this study will provide some information on the need for the second treponemal test among patients with initially discordant syphilis serology results, based on the RPR titer.

1.1.1 Epidemiology of syphilis

Syphilis cases continue to rise globally. In 2012, World Health Organisation (WHO) estimated 18 million prevalent syphilis cases worldwide, with 6 million new syphilis cases among adolescents and adults aged 15–49 years, accompanied by 350000 adverse pregnancy outcomes.[16] In the United States, syphilis incidence has returned to levels not seen in more than 20 years, and the number of cases reported increased by 81% between 2014 to 2018. [17,18]

In Malaysia, the number of acquired syphilis rise more than double from 847 in 2010 (incidence rate 2.99/100 000) to 1854 in 2015 (incidence rate 6.0/100 000),

and a similar trend was observed for congenital syphilis cases.[19] Published data on syphilis prevalence in our country was limited. Old data from 2001 reported syphilis prevalence was 7.33%.[20] A recent study on the seroepidemiology of syphilis in Kuala Lumpur found more than 60% of syphilis cases were among the male gender. The highest percentage was for the young adult population.[21]

In fact, at the end of the 1990s, the occurrence of syphilis declined in several countries with endemic syphilis, primarily due to the implementation of syndromic management for sexually transmitted infections, sexual behavioural changes, and the effects of Acquired immunodeficiency syndrome (AIDS) mortality on sexual networks.[1,22] However, following the introduction of antiretroviral therapy, syphilis rates have increased, presumably due to the reconstruction of sexual networks and increased frequency of sexual contact.[1,5] The high risk groups like men who have sex with men (MSM), transgender women and sex workers are remarkably burdened with syphilis in all regions of the world.[1,4]

Due to the upward trend of syphilis, screening algorithm with high sensitivity and specificity should be available to detect syphilis accurately.[22] The timely diagnosis and proper antimicrobial treatment can lead to significant health benefits in persons who are at increased risk for syphilis by curing the infection, preventing manifestations of late-stage disease, and preventing transmission to others.[9,22,23]

1.1.2 Microbiology

In 1905, Schaudinn and Hoffmann described the causative agent for syphilis, *Treponema pallidum* subspecies *pallidum*. It is a spirochete bacterium measuring approximately 0.2 μm in diameter and 6 to 20 μm in length.[24] Until now, this organism cannot be reliably cultivated on artificial media.[13,24,25] Besides, it is too

slender to be observed using direct light microscopy and is best visualized with darkfield or phase-contrast microscopy. [1,7,24]

Other members of *Treponema* genus that can infect humans are *T. pallidum* subsp. *pertenue* (yaws), *T. pallidum* subsp. *endemicum* (bejel or endemic syphilis), and *Treponema carateum* (pinta).[24] Morphologically indistinguishable, these pathogenic treponemes can induce antibodies which are detected to diagnose venereal syphilis by the routine serologic tests.[24] The distinction among the underlying infections is dependent on geography, clinical manifestations, patient age and other demographic characteristics.[24] Recently, the discovery of the unusual genetic signature of tpp15, a 5′-flanking region of the 15 kDa lipoprotein gene, can be used to differentiate between the strains.[1]

It was assumed, for many years, that *T. Pallidum* has a coating of serum proteins and mucopolysaccharides that protects it from the immune system of the host. The absence of proteins and pathogen-associated molecular patterns (PAMPs) on the spirochetal surface is now generally recognized as the basis for the remarkable ability of the bacterium to immune evasion, which has gained the name "stealth pathogen" and contribute to the chronicity of the disease.[7,24]

1.1.3 Transmission of syphilis

Most cases of syphilis are attributable to sexual contact; therefore, it is considered a sexually transmitted infection (STI).[26,27] Syphilis is transmitted from person to person through direct contact with a syphilitic chancre. Chancres occur primarily on the external genitals, vagina, anus, or rectum but can also appear on the lips and in the mouth; thus, transmission occurs during vaginal, anal, or oral sex.[12] Rectal and oral transmission is frequent in MSM.[5,12] Cases of syphilis in MSM are

of significant concern as early syphilis lesions raise the risk of contracting and transmitting human immunodeficiency virus (HIV) infection.[4,7]

Syphilis may also be transmitted congenitally when spirochetes pass through the placenta of infected women and infect the fetus, in the uterus or during birth.[1,6] Adverse effects are worse in newborns whose mothers have syphilis but have not been treated than those born to mothers who have received treatment.[28,29] Syphilis could also be transmitted through infected blood via sharing needles or rarely via blood transfusion.[5] Infections have also been reported through contact with open lesions, organ transplantation, or occupational and other exposures.[1,2,5]

1.1.4 Clinical spectrum of syphilis

If undiagnosed or inadequately treated, *T. pallidum* subsp. *pallidum* can remain in the body for a lifetime. The disease will progress into stages: early syphilis, which is further divided into primary syphilis, secondary syphilis, and early latent syphilis, whereas late syphilis comprises late latent syphilis and tertiary syphilis. Early syphilis is defined as infection for less than two years, while late syphilis is the disease's occurrence for two years or more.[16]

Once the initial contact with the skin or mucous membranes occurred, the spirochetes will replicate locally, eliciting an inflammatory response and spreading through the blood vessels and lymphatics. Three weeks after exposure, a distinctive painless, ordinarily solitary, clean-based, indurated ulcer (chancre) generally emerges.[2,5,16] The ulcer starts to heal within a few days in penicillin-treated individuals. While, in untreated individuals, the primary lesions will spontaneously heal within 3-6 weeks without scarring. At this time, the spirochetes spread from the

primary site of infection to several organ tissues, primarily the skin, setting a new stage known as secondary syphilis.[2,16]

Secondary syphilis has widespread mucocutaneous lesions that affect both the skin and mucous membranes. Secondary syphilis rash can resemble other infectious or non-infectious conditions, but the palms and soles are characteristically affected, mostly distributed symmetrically and non-itchy. It can have several manifestations or may be mild enough to be ignored. In warm and moist body areas, such as the anus and labia, large white or grey raised lesions will develop due to the spread of the primary lesion's treponemes. Though without treatment, the symptoms and signs of secondary syphilis will spontaneously resolve, and if left untreated, the patient will enter the latent stage.[16]

In latent syphilis, the patient is asymptomatic and only characterized by positive syphilis serology. Left untreated, most patients will remain at this stage, and after 30 years or more of infection, approximately 25% of cases will develop the late clinical sequelae of tertiary syphilis. It can affect any organ system, but the main manifestations usually involve neurological, cardiovascular, and gummatous lesions.[16]

The primary and secondary stages are the most infectious.[7,18] Sexual transmission usually occurs during primary, secondary, or early latent infections; however, the mother-to-child transmission can occur at all syphilis stages, but the risk is higher with early syphilis than later stages disease.[16,17,26]

Due to its versatile presentations and the unpredictable natural history of both untreated and treated disease, the recognition of syphilis can be very challenging even to an experienced clinician.[17]

1.1.5 Laboratory diagnosis

The available laboratory tests for syphilis diagnosis include direct detection methods [i.e. dark field microscopy, direct fluorescent antibody (DFA) test, and nucleic acid amplification test (NAAT)], serology and analysis of cerebrospinal fluids (CSF).[16]

Though dark-field microscopy can demonstrate treponemes with a characteristic morphology, its availability is increasingly limited as it requires specialised equipment and a trained, experienced microscopist.[13] Meanwhile, the DFA test uses a fluorescence microscope to detect spirochetes that have been stained with fluorescein-labelled anti-*T. pallidum* globulin. However, specialised equipment is needed, and the specific fluorescein conjugate is not widely available. NAAT can directly detect *T. pallidum* DNA by polymerase chain reaction (PCR) unfortunately commercial PCR tests for *T. pallidum* are not yet commercially available and is thus relatively expensive compared to other tests used to diagnose syphilis.[3,16,25]

Nowadays, in most laboratory settings, serology is still the most optimal method for syphilis screening and diagnosis.[17,25,27] Although imperfect, serologic syphilis screening is highly sensitive and specific in high-prevalence populations, is inexpensive and technically simple, and has minimal potential for harm.[4,22]

1.1.5.1 Syphilis serology

There are two types of serological testing for syphilis: nontreponemal and treponemal tests.[28] A positive result from both tests is required to maximise the sensitivity and specificity for the diagnosis of syphilis.[25,29]

The most widely used nontreponemal tests are microscopic Venereal disease research laboratory (VDRL) and the macroscopic rapid plasma reagin (RPR).[2,27]

Normally, they are easy-to-use, inexpensive and widely available but requires experienced laboratory personnel to produce subjective results.[13,15,28] These tests detect antibodies formed in response to cellular damage, which can also be produced in other diseases such as acute febrile viral infections and some chronic autoimmune disorders, making it not specific for syphilis and can give false-positive results.[3,13,16] Most commonly, false-positive nontreponemal tests results have titers of less than 1:4.[16]

Nontreponemal tests can be affected by syphilis stages.[3] It may be negative for up to four weeks after the first appearance of primary syphilis lesions as well as in the late syphilis. Also, during primary and secondary stages, these tests may be falsely negative due to a prozone effect.[3,16] That is why, when the suspect lesion is present, repeated testing at two and four weeks may be required to exclude syphilis. A negative nontreponemal test almost excludes the diagnosis of syphilis at three months after the appearance of the primary chancre.[16]

Nontreponemal testing can be qualitative or quantitative. Quantitative test titers may be used to evaluate active syphilis infection and response to treatment.[2,25,28] In untreated active disease, the titer will increase, and with effective treatment, the titers are expected to decrease. A four-fold or greater change in titer, equivalent to a change of at least two dilutions, is considered to be a significant difference between two sequential testing using the same procedure (e.g. VDRL or RPR) and ideally the same laboratory. Titers that differ by only one dilution is not regarded as significant and can only reflect variations in laboratory interpretation.[16]

Meanwhile, the treponemal test which comprises Treponema pallidum haemagglutination assay (TPHA), Treponema pallidum particle agglutination assay (TPPA) and the fluorescent treponemal antibody absorbed (FTA-ABS) are highly

specific because they detect antibodies against treponemal-specific antigens and will remain positive for the patient's lifetime, regardless of treatment and not affected by syphilis stages.[13,15,30] Besides, the treponemal antibody appears earlier than the nontreponemal antibody, starting approximately one week following infection; thus, these tests can detect very early syphilis infections. [14,30,31]

However, treponemal tests cannot differentiate venereal syphilis from endemic syphilis (yaws and pinta) and cannot distinguish between active and previously treated infections.[24] Plus, they are more expensive, and the manual tests are labour intensive compared to nontreponemal tests.[2]

Presently, diverse automated treponemal specific immunoassays are increasingly being used for syphilis screening and diagnosis, including enzyme immunoassays (EIAs), chemiluminescence immunoassays (CIA), microbead immunoassays (MBAs), and many others. [15,32]

1.1.5.2 Algorithms for syphilis diagnosis

Both nontreponemal and treponemal tests have limitations. Thus it is not ideal to use them in isolation for syphilis diagnosis. There are two commonly used algorithms to approach the serologic diagnosis of syphilis: traditional and reverse algorithms, which differ by the sequence in which the tests are performed. [13,17,28] The traditional algorithm starts with a nontreponemal test, and if reactive, a treponemal test will follow for confirmation.[28,32,33]

Since the last decade, there has been an increase in the adoption of automated treponemal tests for syphilis screening, resulted in the syphilis testing algorithm being reversed.[12,13,25] In the reverse algorithm, reactive treponemal test screening will be followed by a quantitative nontreponemal test for diagnosis confirmation. In case of

discordant results obtained between the treponemal screen and the nontreponemal test, second confirmatory treponemal test (e.g., TPPA) should be performed, which preferably detects different antigens than the treponemal screen. [28,32,33]

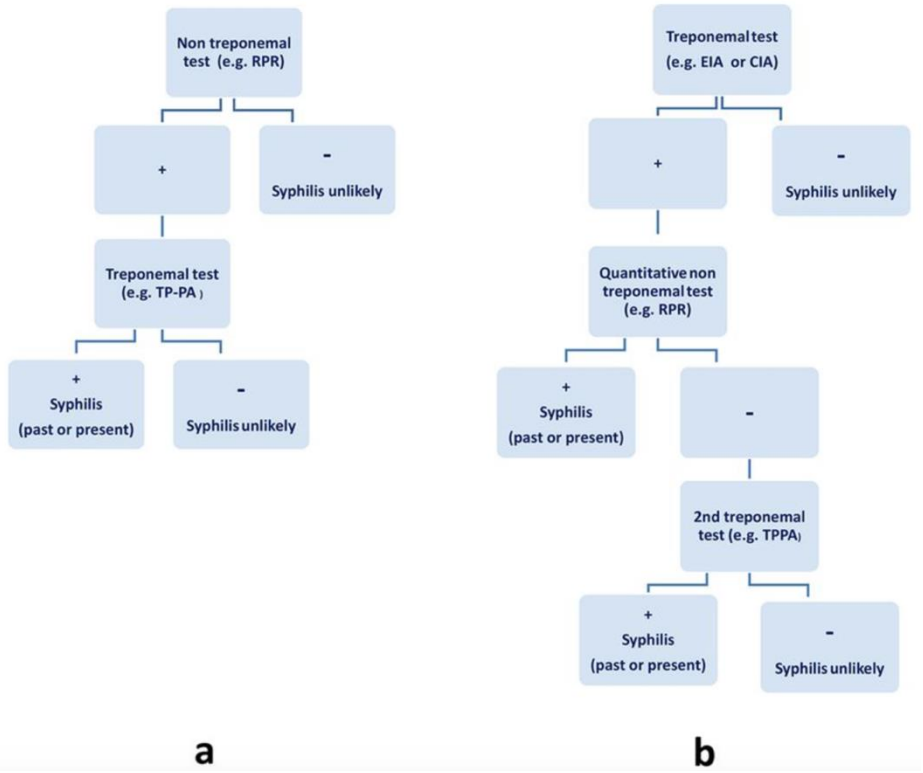

Figure 1.1: Testing algorithms for syphilis diagnosis. (a) Traditional algorithm. (b) Reverse algorithm. +, positive result; −, negative result; RPR, rapid plasma reagin; TP-PA or TPPA, *Treponema pallidum* particle agglutination test; EIA, enzyme immunoassay; CIA, chemiluminescence immunoassay [Adopted from Morshed MG, Singhb AE. Recent trends in the serologic diagnosis of syphilis. Clin Vaccine Immunol. 2015;22(2):137–43 (34)]

In the reverse algorithm, an analytical false positive can be considered when a reactive treponemal screen with non-reactive nontreponemal and negative second treponemal confirmatory testing. A reactive both screening and second treponemal tests but non-reactive nontreponemal test may be an analytical false-positive treponemal tests due to cross-reactive antibodies or an analytical true positive result in the late or previously treated syphilis.[13]

The quantity of syphilis testing mainly drives the algorithm used. Most nontreponemal tests use manual assays.[15] Therefore, high-volume laboratories have opted to follow the reverse algorithm due to the availability of United States Food and Drug Administration (FDA)– cleared, automated treponemal platforms that operate high throughput testing with objective results.[15,23,35]

Reverse algorithms could detect current infections and past infections that would have been undetected by the traditional algorithm.[14,25] While the reverse algorithm is more timely and cost-effective, it does have a 14–40% false-positive rate, requiring a second treponemal test for syphilis confirmation, thus lead to inefficiencies in the laboratory.[12–14,23] Several analyses have been performed and concluded that treponemal immunoassay signal strength values might be used in place of the second treponemal test, thus avoiding additional costs and shorten time-to-result.[14,15]

Overall, a decision on using the reverse algorithm is to be determined based on a combination of local syphilis prevalence, expected workload, the requirement of automation, and budget.[13,33] Unfortunately, there is no gold standard for serological syphilis testing, and therefore, all screening results must be correlated with the clinical presentation for the diagnosis of syphilis.[23,27,35]

1.1.6 Syphilis treatment

Penicillin is a drug of choice and highly effective at all stages of syphilis. [9,17] Penicillin resistance has not been observed in *T. pallidum*. A dose of 2.4 million units of long-acting benzathine penicillin G administered intramuscularly sustains blood levels of treponemicidal for 7 to 10 days and effectively treats uncomplicated early syphilis.[17,36,37] Three doses of benzathine penicillin G, administered at weekly intervals, are given for late latent syphilis. While the optimal interval between doses

is seven days, up to 10 days between doses may be appropriate in nonpregnant adults.[17]

Patients with neurosyphilis are treated with intravenous aqueous penicillin G due to low concentrations of benzathine penicillin G in the CSF.[17,36] In cases with known penicillin allergy, desensitisation and subsequent treatment with penicillin are recommended. Restricted data limits the use of substitute antibiotics, which should only be used when treatment with penicillin is not feasible or is contraindicated.[17,38]

With effective treatment, the serologic cure is expected 6 to 12 months after therapy for early syphilis and 12 to 24 months for late syphilis.[17]

1.2 Rationale of the study

1. **Different screening tests and algorithms used for syphilis diagnosis between different hospitals in the population.**

   Hospital Universiti Sains Malaysia (HUSM) has adopted automated treponemal test using ECLIA for syphilis screening, meanwhile many other hospitals in Malaysia are still using a nontreponemal test (RPR) for syphilis screening. ECLIA and RPR represent the reverse and traditional screening algorithm, respectively. Reverse screening algorithm was known to have higher sensitivity as compared to the traditional algorithm. Comparison of accuracy between these tests might reflect the usefulness of reverse screening algorithm for the higher detection rate of syphilis in our community.

2. **Unavailable optimal cut-off for RPR titer to predict true-positive syphilis infection**.

   Among the limitations of RPR is the lack of specificity for syphilis infection. RPR reactivity can be due to conditions other than syphilis infection, such as biological false-positive. In early syphilis infection, RPR titer will increase and can be used to monitor disease activity. On the other hand, RPR titer will be reduced by effective treatment and can be affected by stages of syphilis infection. The previous study has shown that the higher RPR titer was associated with an increased probability of positive confirmation for syphilis. Determination of optimal cut-off titer for RPR will guide the need for additional testing for syphilis diagnosis when using the reverse algorithm.

3. **Lack of data on epidemiology of syphilis in the population.**

   This study may provide estimation for the proportion of syphilis among screened patients in HUSM, with information on gender and age distributions of patients infected with syphilis.

1.3 Study flowchart

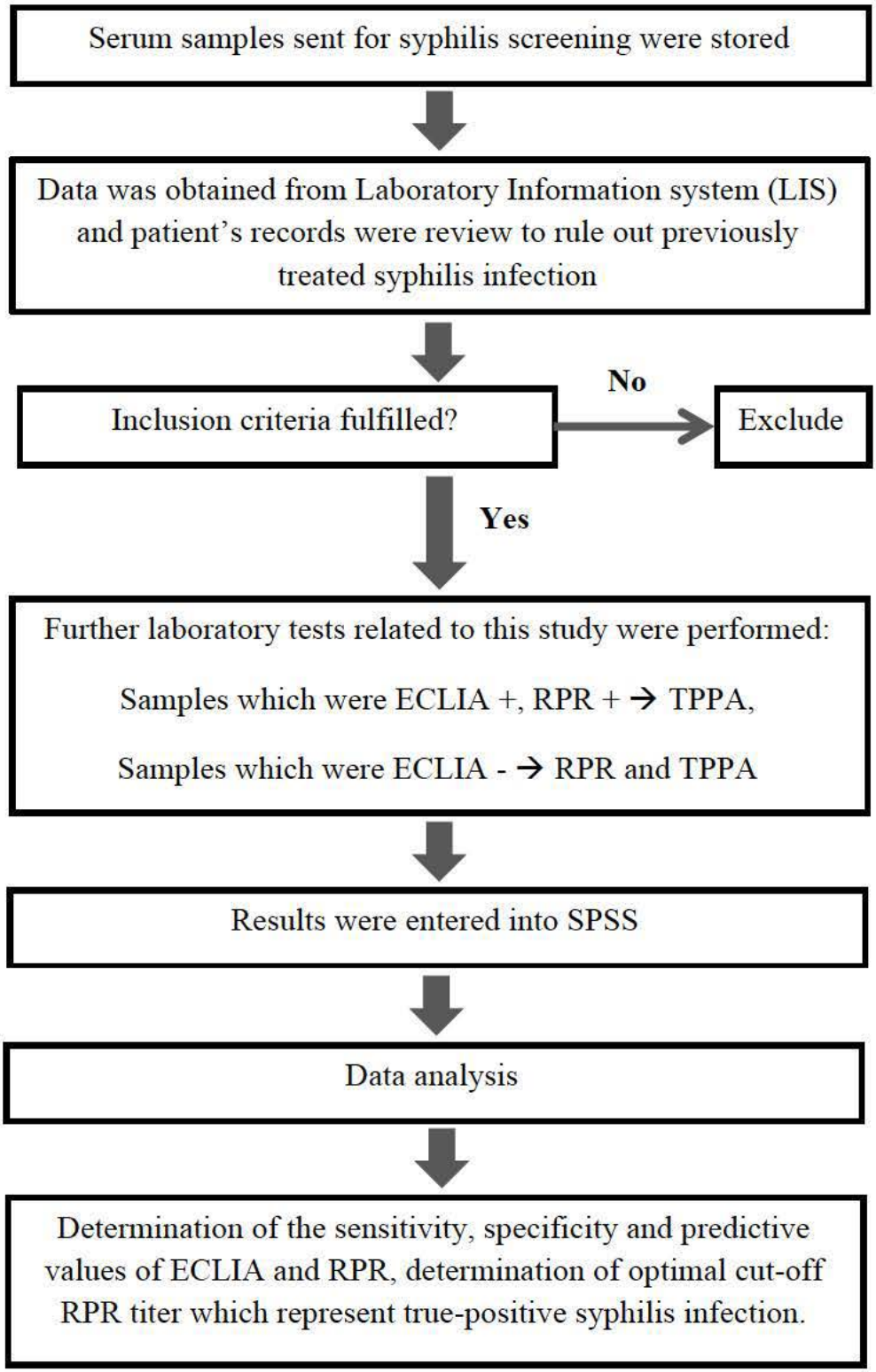

Figure 1.2: Study flowchart. ECLIA, electrochemiluminescence immunoassay; RPR, rapid plasma reagin; TPPA, *Treponema pallidum* particle agglutination; +, reactive; -, non-reactive; SPSS, Statistical package for the social sciences.

1.4 Research Question(s)

1. What is the proportion of syphilis among screened patients in HUSM?
2. What are the sensitivity, specificity and predictive values of ECLIA and RPR in relation to TPPA?
3. What is the optimal cut-off RPR titer to predict true-positive syphilis infection?

1.5 Objectives

General: To evaluate the diagnostic accuracy of screening tests used in syphilis investigations.

Specific:

1. To estimate the proportion of syphilis among screened patients in HUSM.
2. To determine the sensitivity, specificity and predictive values of ECLIA and RPR in relation to TPPA.
3. To determine the optimal cut-off RPR titer to predict true-positive syphilis infection.

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CHAPTER 2: STUDY PROTOCOL

2.1 Title

Comparison between reverse and traditional screening algorithms for syphilis diagnosis in Hospital Universiti Sains Malaysia.

2.2 Objectives

2.2.1 General objective

To evaluate the diagnostic accuracy of screening tests used in syphilis investigations.

2.2.2 Specific objectives

1. To estimate the proportion of syphilis among screened patients in HUSM.
2. To determine the sensitivity, specificity and predictive values of ECLIA and RPR in relation to TPPA.
3. To determine the optimal cut-off RPR titer to predict true-positive syphilis infection.

2.3 Methodology

2.3.1 Study design

This is a hospital-based cross-sectional study involving serum samples which were sent for syphilis screening conducted from January 2020 to June 2020.

2.3.2 Reference population

Serum samples which were sent for syphilis screening in Kelantan.

2.3.3 Target population

Serum samples which were sent for syphilis screening in HUSM.

2.3.4 Source population

Serum samples which were sent for syphilis screening in HUSM from January 2020 until June 2020.

2.3.5 Sampling frame

Serum samples which were sent for syphilis screening which meet the inclusion and exclusion criteria.

2.3.6 Inclusion criteria

Serum samples sent for syphilis investigations.

2.3.7 Exclusion criteria

1. Serum samples sent for monitoring of treatment response to syphilis.
2. Serum samples requested for RPR only.
3. Defected serum samples or insufficient volume for sample analysis.
4. Serum samples which shown indeterminate results for either tests.

2.3.8 Sample size estimation

Objective 1

For objective 1, sample size estimation was calculated using single proportion formula.[1]

1 proportion – Estimation		
Proportion (p)	7.33%	
Precision	6.00%	
Significance level (α)	0.050	Two-tailed
Drop-out	10%	
Sample size	73	
Sample size (with drop-out)	82	

Figure 2.1: Sample size calculation using single proportion formula.

The proportion (p) included in this formula is 7.33% based on syphilis prevalence reported by Malaysia Consensus Report on STI, HIV and AIDS Epidemiology, 2001.[2]

Objective 2

For objective 2, sample size estimation was calculated using sample size calculator for sensitivity and specificity.[1]

1) For ECLIA, the expected sensitivity and specificity were 100% and 99.8% respectively.[3]

Sensitivity/Specificity – Estimation		
Expected Sensitivity	100.00%	
Expected Specificity	99.85%	
Prevalence of disease (p)	7.33%	
Acceptable precision (W)	6.00%	
Significance level (α)	0.050	Two-tailed
Drop-out	10%	
Sample size for Sensitivity	0	
Sample size for Specificity	2	
Final Sample size	2	
Sample size (with drop-out)	3	

Figure 2.2: Sample size calculation based on expected sensitivity and specificity for ECLIA.

2) For RPR, the expected sensitivity and specificity were 100% and 80.8% respectively.[4]

Sensitivity/Specificity – Estimation		
Expected Sensitivity	100.00%	
Expected Specificity	80.80%	
Prevalence of disease (p)	7.33%	
Acceptable precision (W)	6.00%	
Significance level (α)	0.050	Two-tailed
Drop-out	10%	
Sample size for Sensitivity	0	
Sample size for Specificity	179	
Final Sample size	179	
Sample size (with drop-out)	199	

Figure 2.3: Sample size calculation based on expected sensitivity and specificity for RPR.

From the above calculations, the largest sample size after considering 10% drop-out was **199**. We managed to tests **206** samples in this study.

2.3.9 Sampling method

Convenient sampling was used as the sampling method. All serum samples for syphilis screening which fit the inclusion and exclusion criteria were recruited in the study.

2.3.10 Operational definition

1. Interpretations of laboratory tests results:

   i. For ECLIA, the reactive cut-off index (COI) is considered when COI ≥1.00 and non-reactive COI is when COI < 1.00, following the COI provided by the manufacturer.

   ii. For RPR, reactive reaction is with the presence of characteristic clumping ranging from slight but definite (minimal-to-moderate) to marked and intense. When quantitative RPR is performed, the report is provided in terms of the